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THE QUESTION OF THE TOXICITY OF DISTILLED WATER *

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INTRODUCTION

Attention has recently been drawn to the necessity of a physiologically balanced salt solution for water cultures. This necessity arises from the fact that in order to know the effect on organisms of certain single salts or mixtures of salts in solution, one must use a control or check solution, called by True,[1] a normal physiological solution. This solution or medium should cause no disturbance of the regular functions of the seedling. Such a culture would afford an ideal standard for comparison and one much to be desired. The usual method, however, has been to use distilled water. That such cultures for biological studies are unsuitable was shown some time ago.

In the first place, 'distilled water' is an indefinite term. As soon as the seedling roots are introduced the conductivity of the water rises rapidly, showing that salts are leached from the roots and the water remains no longer pure. Secondly, the extraction of salts, it is affirmed, suggests the means by which pure samples of distilled water exert their harmful effects. Some authors affirm a starvation theory; others, like Loeb,[2] the loss of certain necessary ion-proteid compounds; and still others, as True,[3] that certain necessary constituents, partly inorganic, are dissociated from their proper attachments in the complicated chemical and physical mechanism of the living cell. Thirdly, the harmfulness of distilled water is attributed by others to the presence in it of toxic substance derived from the apparatus or because of the method of preparation.

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* Received for publication April 10.

[1] True, R. H. Distilled Water in the Laboratory. Science n. ser. **39**: 296. 1914.

[2] Loeb, J. On Ion-proteid Compounds and Their Rôle in the Mechanics of Life Phenomena. I. The Poisonous Character of a Pure NaCl Solution. Amer. Journ. Physiol. **3**: 327. 1899–1900.

[3] True, R. H. The Harmful Action of Distilled Water. Amer. Journ. Bot. **1**: 270. 1914.

The two views, namely, that the distilled water is toxic *per se*, or that it is toxic because of the presence of poisonous substances in it, have been the principal ones held to the almost complete exclusion of any other. In this paper the writer wishes to draw attention to two other aspects of the distilled water problem. It seems that the problem is more a dynamic one than otherwise and in all probability more than one factor enters into the explanation of why seedlings cannot survive in distilled water. Right at this point it must be said that certain seedlings because of various physiological characteristics are less susceptible to distilled water injury than others. It is very probable that different varieties of seedlings and even different individuals of the same variety react differently to the same external conditions. From the work done in this laboratory but not reported the conclusion can be drawn that, providing one selects disease-free seed uniform in size, color, etc., one can detect as much individual variation in the behavior towards toxic substances in solution of seedlings of pure line seeds from one plant as in that of seedlings of a mixed progeny from unknown source. It follows from this that a medium which affords optimum conditions for one organism will not be the best for another. A normal physiological solution as mentioned above will perhaps afford optimum conditions for one kind of seedlings but not for another. Certain other salts or other proportions are better. In all probability there is no universal physiologically balanced solution. Loeb[4] states that salt water is a physiologically balanced salt solution for *such organisms as thrive in it* and the blood of normal, healthy subjects can also be considered a physiologically balanced solution. From our knowledge of balanced solutions it may be assumed that the rôle of the various salts is one of antagonistic action, a neutralization of the toxic effect of a single salt or of the harmful proportion of the various salts in the medium. That distilled water cultures of seedlings are not physiologically balanced solutions, can be assumed without much danger of contradiction. Wherein, then, lies the inappropriateness of distilled water for control cultures; and wherein lies the explanation of the injurious effect of such waters, and still further injury as the seedlings continue to grow in it? This preliminary paper will endeavor to show, *first*, that the adjustment of the seedlings to a distilled water medium is an important factor. It seems probable that injury is due to the greatness of the change. It will attempt to show *secondly*, that the possible injury which might arise from toxic substances excreted by the roots while the seedlings are growing in the water is another important factor to be considered.

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[4] Loeb, J. The Relative Toxicity of Distilled Water, Sugar Solutions and Solutions of Various Constituents of the Sea Water for Marine Animals. Univ. Calif. Publ. 1: 62 and 69. 1903.

In lieu of any historical resumé, mention is here made of the literature and references cited by Livingston,[5] Hoyt[6] and True.[7] These papers give a very complete summary of the history of the work done up to date by both animal and plant physiologists. It is interesting to note that the work is about evenly divided between these two divisions of biology.

Materials and Methods

The distilled water used in these experiments was of low conductivity, $2.5 - 1.4 \times 10^{-6}$. A battery of stills was set up and kept in operation all day and on the following days until the required amount of water was obtained. Then the experiment was started. The waters were stored in clean Jena bottles for no longer than the period of experimentation. Each individual still consisted of one 3-liter Jena glass flask, one condenser with spiral Jena glass inner tube, Jena glass tubing for connections where necessary and cotton plugs. The water was collected in either 5-liter, ground-glass-stoppered Jena bottles, or 3-liter ground-glass-stoppered Jena flasks.

As indicators, the roots of *Lupinus albus* were selected. The seeds were kindly given me by Dr. True. These were soaked over night in either tap water or distilled water and suspended in damp chambers so as to form straight roots. No trouble was experienced in obtaining straight roots. When of the proper length the roots were marked at a point 10 or 15 mm. behind the tip and inserted in the loop of paraffined copper hooks. These were never allowed to touch the water, and only the radicles were immersed. These hooks were then arranged around the rim of Jena beakers of 250 cc. capacity. All glassware was previously thoroughly washed, rinsed, soaked in sulphuric acid-potassium dichromate solution for a few hours, then washed, thoroughly steamed and finally rinsed with the test water before being used in the experiment. The growth rate was chosen as the criterion. The control solution was tap water. This was allowed to run freely from the faucet for at least 15 minutes so as not to use the water that had rested in the supply pipes. It was then collected in clean Jena glass flasks and put aside near a radiator to come quickly to the room temperature, the temperature at which the distilled water was maintained. The cultures were set aside on a table away from the windows and covered with paper.

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[5] Livingston, B. E. Further Studies on the Properties of Unproductive Soils. U. S. Dept. Agr., Bur. Soils Bull. **36**: 57, 71. 1907.

[6] Hoyt, W. D. Some Toxic and Antitoxic Effects in Cultures of Spirogyra. Bull. Torrey Club **40**: 333–360. 1913.

[7] True, R. H. The Harmful Action of Distilled Water. Amer. Journ. Bot. **1**: 253–273. 1914.

PREPARATION OF DISTILLED WATER

Only water which was prepared in contact with laboratory air of average purity is to be here considered. Absolute water prepared and used in vacuo[8]; water obtained by the combination of oxygen and hydrogen in the presence of an electric spark[9] or water distilled from a quartz distilling apparatus in contact only with air from out-of-doors dried and rid of CO_2 by the usual methods,[10] is not here used. A comparative study of all the available methods was made. Various combinations were used for the purpose of eliminating this or that source of trouble. In all twenty-six methods were tried. Livingston[11] showed that good water could be obtained by shaking distilled water with finely divided carbon black. By a process of adsorption both volatile and non-volatile substances toxic in nature are taken up. E. P. Lyon[12] obtained water of excellent quality by adding a little H_2SO_4 to the tap water in an automatic still consisting of a copper vessel and glass condenser, providing none of the condensed water touched the metal. It is suggested that the volatile toxin, ammonia, is eliminated by the H_2SO_4 treatment. Hoyt[13] made an improvement on Livingston's method by distillation with animal charcoal in the retort. A combination of the last two with an additional treatment mentioned below gave the best water in contact with air that the writer could get and one that had a conductivity of 1.4×10^{-6}.

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[8] Kohlrausch & Heydweiller. Wied. Ann. **53**: 209. 1894.

[9] Any of the good text-books on general chemistry.

[10] Method used in the research laboratory of physical chemistry, University of Illinois. Not yet reported.

[11] Livingston, B. E., Jensen, C. A., Breazeale, J. F., Pember, F. R., Skinner, J. J. Further Studies on the Properties of Unproductive Soils. U. S. Dept. Agr. Bur. Soils Bull. **36**. 1907.

[12] Lyon, E. P. Biological Examination of Distilled Water. Biol. Bull. Marine Biol. Lab. **6**: 198. 1904.

[13] Hoyt, W. D. Some Toxic and Antitoxic Effects in Cultures of Spirogyra. Bull. Torrey Club **40**: 338. 1913.

About 5 liters of tap water were first shaken up with animal charcoal and allowed to stand, with frequent shakings, for several hours, and then transferred to the retort for distillation. This length of time is hardly necessary since the adsorption equilibrium is reached in a comparatively short time. The first 500 cc. distilled over was discarded and the last 800 cc. was not distilled. The part left, the middle portion, was poured into a second retort to which a little concentrated H_2SO_4 was added and distillation started. The first 300 cc. (about) was discarded and the last 800 cc. not distilled. The middle portion proved to be a medium in which lupine seedlings grew very well and it was thought, since previous work had shown that dilute solutions of H_2SO_4 stimulated growth, that some sulphates might have passed over. Three tests were made for sulphates but none were found. It was thought that here was a suitable water, but to allay all suspicions it was again distilled in a third retort to which was added a little $Ba(OH)_2$. The middle portion from this distillation was caught in a 5-liter Jena bottle, thoroughly cleaned and steamed. The water was then aerated. The air was drawn from out-of-doors through tubes of calcium chloride, sulphuric acid and potassium hydroxide solution. This bubbling process was continued for about twenty-four hours. In this water, lupine seedlings made good growth and showed not the least sign of injury for three weeks providing the distilled water was changed at least twice daily.

In the following experiment the records were kept for a week. The experiment was continued, however, and at the end of three weeks the seedlings were in good condition but not as far advanced as the checks in tap water. There were 10 seedlings in each beaker. At the end of the first twenty-four hours the total average growth in distilled water was 33.7 mm. On the following day the average increase was 4.5 mm. The increase for each following day is 3.6 mm., 5.3 mm., 4.3 mm., 7.8 mm. and 7.3 mm. The data for tap water follow: At the end of 24 hours the total average for 10 seedlings was 34.6 mm. Increase during successive 24-hour periods: 11.7 mm., 15.2 mm., 13.5 mm., 9.6 mm., 11.6 mm., 9.1 mm. It may be noted here that the growth is a little better in tap than in distilled water, but the rate of increase is of the same order. The growth in tap water is quite luxuriant. Analysis of the tap water kindly given me by the station chemist, Prof. A. J. Patten, is here appended.

Total Solids	CaO	MgO	SO_3	Cl
Parts per 100,000	33.9	11.06	00	.025

THE PROBLEM OF ADJUSTMENT

It seems that marked and sudden changes from waters of low resistance to waters of high resistance would affect the equilibrium between the physical and chemical mechanisms of the living cell. When the changes are not so great the deleterious effects will not be so great and the organism can survive in a medium, in which, if thrust suddenly, it would die. This fact also suggests another generally admitted but so often overlooked in biological studies. If comparative results are desired, the organisms used must be living under uniform conditions previous to the time of experimentation. Their future behavior depends as much on their internal nature, which is the result of their previous surroundings and past history, as on the condition of the surroundings into which they will be placed. One will not be offering a too daring interpretation if he regard this feature of adjustment as a very important one in the consideration of the injurious effect of distilled water. My experiments agree in general with the results obtained by Daniels[14] who concluded that death of Paramecia in distilled water was caused by the sudden change in media.

Peters states, "that if a change from culture liquid to pure water be made as rapidly as in these experiments (with Stentor and Paramecia) a fatal effect ensues. On account of the conception I have formed of the mode of action of the pure water, I would expect the same effect to occur even if the changes occupied a longer period of time." The inference is that the factor of adjustment is not important.

To test this theory of adjustment for lupine seedlings and distilled water, various mixtures of tap and distilled water were made. The seedlings were first placed in a beaker of tap water and allowed to grow in that for a definite time. Then all but 5 were removed and placed in a beaker containing a mixture of tap water and distilled water in the ratio of 1 : 4. After a definite time all but 5 of these were removed and placed in a mixture in the proportion of 1 : 8. This operation was repeated until 5 were placed in distilled water from the last dilution of 1 : 64. The experiment was then allowed to run 24 hours longer. Now the total growth was recorded in each beaker. At the same time that this series was set up a duplicate one was arranged and placed on the table beside the first. At the beginning of the experiment 5 seedlings were placed in each beaker. The total growth of the roots of these seedlings was recorded at the same time as the other set. In the last beaker of the first set were five seedlings that had been placed in the distilled water after they had been growing for a stated interval in 5 different dilutions of distilled and tap water. In the last beaker of the second series were five seedlings that had been immediately placed in distilled water. In this way one set of seedlings was exposed to the influence of distilled water after gradual changes and the other immediately. In another experiment the method was varied, in one way, by using mixtures containing different ratios of tap and distilled water, and in another way by arranging the experiment so that 10 seedlings could be put into the last beaker of the series. The change was first made daily, in another experiment twice a day, and in the final experiment four times a day. In one experiment the records of root growth were made at every change in every beaker. The results of these experiments show that better root growth was obtained in distilled water when changes from tap to distilled water were made gradually instead of suddenly. In the following table T and D stand respectively for tap and distilled water.

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[14] Daniels, J. F. The Adjustment of Paramecia to Distilled Water and Its Bearing on the Problem of the Necessary Inorganic Salt Content. Amer. Journ. Physiol. 23: 48–63. 1908–9.

Where Changes are Sudden

	Tap	$\frac{1}{4}T+\frac{3}{4}D$	$\frac{1}{16}T+\frac{15}{16}D$	$\frac{1}{32}T+\frac{31}{32}D$	$\frac{1}{64}T+\frac{63}{64}D$	Dist.
Total growth in mm.....	34.3	36.5	37.4	31.2	31.5	27.5
Where Changes are Gradual						
Total growth in mm.....	38.3	43.4	38	36.4	36.3	34.3

Greater differences might have been obtained had a more sensitive indicator been selected. As far as our experience goes lupines are not very sensitive to distilled water injury. When exposed to sudden changes they react in a moderate way. When in distilled water frequently changed to rid the medium of the toxic substances or unbalanced condition of the organic and inorganic material therein, they live until the nutritive substances in the cotyledons have been exhausted. Frank's[15] statement that lupine seedlings are poisoned by distilled water has been questioned by Schulze[16] who states that in all probability the effect produced is the result of impurities in the water. From our experience with lupines we would prefer to agree with Schulze.

It may also be observed from a study of the experimental data above that the change from the last dilution to distilled water affects the growth rate more than changes in the other dilutions. Were the cause of injury due to osmotic effects, this would show up in the first or greater change but here we always have a better growth rate than in any of the other dilutions. That osmotic effects can be ruled out has been shown by Loeb, True and others. Can the same be said of low salt content? Is the low salt content of the medium the cause of injury? J. F. Daniels[14] showed that the destructiveness of distilled water cannot be due to its low content of inorganic salts. Two drops were diluted with equal amounts of distilled water. Into one having the high original salt content of the culture solution many paramecia were placed, into the other with a very low original salt content few organisms were placed. The paramecia in the first drop died quickly, while in the second drop they lived for days. Obviously the salt content was not the important factor. Here no mention is made of the possible deleterious effects of toxic excreta from so many organisms. If lupine seedlings are grown in distilled water with frequent daily changes the salt content will be kept quite low and in addition the toxic excretions will be more or less eliminated. Under these conditions lupine seedlings grow well until they show signs of injury from lack of nutrient salts. The experiments mentioned above, besides others not reported here, lead us to the belief that the problem of adjustment is so important that it must be taken into account in culture experiments.

THE PROBLEM OF TOXIC EXCRETIONS

For a little more than ten years the Bureau of Soils of the United States Department of Agriculture has sought to explain soil infertility, by the presence of deleterious substances of organic nature in the soils. These substances may be given off by the roots, or arise from

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[15] Frank, B. Untersuchungen über die Ernährung der Pflanzen mit Stickstoff und über den Kreislauf desselben in der Landwirthschaft. Landwirthsch. Jahrb. **17**: 421–553. 1888.

[16] Schulze, E. Über das Verhalten der Lupinenkeimlinge gegen destillirtes Wasser. Landwirthsch. Jahrb. **20**: 236. 1891.

the decay of organic matter. It must be admitted that these investigators have collected a vast amount of facts to substantiate this theory. In Bulletin 23[17] it is shown that the bad characteristics of a soil are transmitted to its aqueous extract. The injury to wheat seedlings grown in this as compared with their growth in an aqueous extract of a good soil, could not be attributed to a difference in nutritive salts for they analyzed practically the same. The conclusion was drawn that the solution contained injurious substances which checked the growth of plants. In Bulletin 28[18] it was shown that wheat seedlings give off substances which are toxic to themselves. This is true when seedlings are grown in nutrient solutions which have been previously used for growing plants. Carbon black or ferric hydrate corrects the used solution so that it produces nearly as good growth as the fresh one. The same is true when distilled water is used instead of the nutrient solution. "These results with distilled water," the author states, "seem to make it very certain that the roots of seedling wheat plants do give off substances which are poisonous to themselves, and these substances can be corrected by carbon black or ferric hydrate." This recalls to mind the experiments by Macaire[19] in 1832, who noted that peas grew poorly in water containing excretions from the roots of the same plants, while wheat on the other hand did well in water charged with the excretions from pea roots. Later, 1845, Boussingault[20] studied the question of root excretion and came to the conclusion that plants do not normally excrete materials from their roots, but may do so in water culture.

For a more extended account of the earlier literature and especially that of the work of the Duke of Bedford and S. U. Pickering on the deleterious effect of grass on fruit trees, the writer wishes to refer the reader to Bulletin 36 by B. E. Livingston, and the works of Schreiner and his assistants, found in bulletins of the Bureau of Soils, U. S. Department of Agriculture, Nos. 47, 53, 70, 74, 75, and 80.

What we are most concerned with at the present time is the possibility of deleterious root excretions from lupine seedlings. We must admit in the beginning that we have not isolated any toxic substance from the water in which these seedlings have been growing. The quantity of poison is obviously so small that it will necessitate the evaporation of 10 or 12 liters of solution to get enough residue for a chemical test. The difficulty of this problem is still further increased since organic chemists can give us no universal method of treatment applicable to all unknown solutions. Previous literature gives us assurance that the excretions are probably organic in nature. The probability is that the substances excreted are compounds of multiple function, and if they do contain an acid radicle, this radicle must be internally compensated with some basic group, since the solution gives no reaction to the ordinary indicators. If an aldehyde is sought for we meet with a difficulty in that we have no differential test to indicate what aldehyde it may be.

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[17] Whitney, M., and Cameron, F. K. Investigations in Soil Fertility. U. S. Dept. Agr. Bur. Soils Bull. 23. 1904.

[18] Livingston, B. E., Britton, J. C., and Reid, F. R. Studies on the Properties of an Unproductive Soil. U. S. Dept. Agr. Bur. Soils Bull. 28. 1905.

[19] Macaire-Prinsep, Memoire pour servir à l'histoire des assolemens; Mém. Soc. Phys. Hist. Nat. Genève, 5: 282–302. 1832.

[20] Boussingault, J. B. J. D. Rural Economy. Trans. by George Law (London), 345. 1845.

On the other hand, we have one or two indirect methods to attack this subject of toxic excretion. After a certain length of time in distilled water lupine roots display certain characteristic features not noticeable in tap water cultures. Roots in tap water are long, slender, grayish white, usually straight and firm. A large conspicuous root cap covers the tip. The water does not become turbid. In distilled water the roots present quite a different appearance. They are covered with a felt-like coating which at first gives the appearance of a mass of root hairs or a weft of fungus mycelium. It is believed that this is nothing more than root cap tissue hanging on the root as the tip pushes forward. On closer examination under the microscope this material does not present the characteristic structure of root hairs or fungus hyphae and resembles in every particular the cells of the outer layer of the root cap. No reason is hazarded for its abundance in distilled water. It has not been noticed in tap water, and appears less abundant in distilled water that has been changed two or three times daily. This weft of tissue falls off and gives the water a turbid appearance. In distilled water the roots are shorter, thicker and usually more or less bent near the tip, showing the tendency for fishhook formation. The lateral roots appear earlier and are more abundant in distilled water cultures than in tap water cultures. They show all the appearances of roots suffering from slight doses of poisons. In all probability the roots excrete a toxin which retards growth. If this toxin is removed by frequent changes of the water the growth rate is increased. This can be shown in gradation. If the water is changed once a day, the growth rate changes but little in the earlier stages of the life of the seedling in the water. If changed twice a day, the growth rate is increased. When changed four times a day the best results were obtained. Again a large number of seedlings growing in the water suffer less apparently than a few in the same amount of water. It seems that the greater amount of salts leached out in one case acts in an antitoxic manner and so corrects the water, or the roots might actually reabsorb some of the salts. Some such explanation might be given for the condition reported by True to the effect "that the leaching process seems to have been somewhat less active in the cultures containing the larger number of roots than in that containing the smaller number of roots." Some such conclusion is more reasonable than to assume that roots under the same external condition do not lose salts at approximately the same rate.

In soils where oxidation is hindered we are told that those substances inimical to growth are more apt to be found. The very nature of water cultures produces this condition of poor oxidation and organic matter collects more rapidly than it can be oxidized. A renewal of the medium in which the plants are growing eliminates to a greater or less extent the toxic substance present therein, and at the same time the roots are living in a solution continually aerated. When an experiment is set up with these facts in mind lupine seedlings will survive in distilled water until injury from lack of nutritive substances sets in. The rate of growth is also greater. Ten seedlings were suspended, as described above, in distilled water. The water

Distilled Water Changed		Distilled Water Not Changed	
Seedling No.	Growth in Mm.	Seedling No.	Growth in Mm.
1	44	1	25
2	34	2	31
3	42	3	31
4	38	4	34
5	32	5	35
6	43	6	37
7	41	7	29
8	37	8	32
9	39	9	36
10	46	10	37
Av. Growth in mm. 39.4		Av.Growth in mm. 32.7	

was changed four times daily. As a control for this experiment 10 seedlings were suspended in another beaker of distilled water. The water in this beaker was not changed. At the end of 30 hours, even, these seedlings surpassed the growth of those left in distilled water without change. This can be seen from the obove table.

The table below gives the data on another experiment extending over a period of four days.

Seedling No.	Distilled Water Changed				Distilled Water Not Changed			
	1st 24 Hrs.	2d 24 Hrs.	3d 24 Hrs.	4th 24 Hrs.	1st 24 Hrs.	2d 24 Hrs.	3d 24 Hrs.	4th 24 Hrs.
	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.	Growth in Mm.
1	27	34	43	46	33	39	43	48
2	34	37	41	45	25	33	40	46
3	37	42	48	55	31	35	36	41
4	33	37	47	55	37	40	43	49
5	32	40	51	58	35	38	43	51
6	35	38	47	55	34	37	42	48
7	31	34	40	45	35	40	43	48
8	37	45	50	60	36	41	45	50
9	38	44	51	61	35	39	41	45
10	38	45	53	60	36	40	42	45
Av. growth in mm........	34.2	39.6	47.1	54.0	33.7	38.2	41.8	47.1

CONCLUSION

In regard to the question as to the toxicity of distilled water, the writer wishes to draw attention to the importance of considering the problem from the dynamic standpoint. To describe how the distilled water produces its effect, it is necessary to know what physical and chemical conditions prevail in the organism and what relation exists between the organism and the medium in which it lives. Our present knowledge of the physical and chemical nature of protoplasm, the basis of all organic life, is too fragmentary upon which to lay the foundation of any theory. However, it seems that one will not stray far away from the truth if he concludes the harmfulness of distilled water due not to any one predominant factor but rather to a resultant of many. These factors may be conceived of as forces bringing about a disturbance of the normal equilibrium of the various chemical and physical interactions within the organism and between it and its environment.

In this paper only two aspects of the subject have been considered.

First, there is the problem of adjustment. Some organisms because of their inherent physiological characteristics possess a low order of resistance to harmful agents and are injured, sometimes fatally, by slight deviations from optimum conditions. However, in the case of lupine seedlings a slow process of adjustment or acclimatization to distilled water fits them better for life within it than if they were thrust into the water immediately. That the change from tap water or culture solution, whatever it may be, to distilled water is great, can be demonstrated by the determination of the electrical conductivity of the waters. It appears that such marked differences disturb the equilibrium between the physical and chemical functions of the organism. Several experiments were made and one was reported to test the theory of adjustment. The conclusion was reached that better root growth was obtained in distilled water when changes from tap to distilled water were made gradually rather than suddenly. This problem of adjustment is so important that it must be taken into account in all cultural work.

The second aspect of the distilled water question dealt with in this paper is that of toxic root excretions. The idea that roots of plants excrete injurious substances, harmful to their further growth, has persisted from the time of De Candolle. The investigations of the Bureau of Soils of the U. S. Department of Agriculture appear to set this theory on a more secure foundation. In this paper are reported the results of experiments to show that, in all probability, seedling roots of *Lupinus albus* excrete a substance that inhibits growth. The toxin was not isolated from the turbid solution but it was shown that when the water was changed four times daily the growth rate of the roots increased. Roots that had grown continually in distilled water presented the appearance of roots injured by dilute solutions of toxic salts. The roots were thick, short, more or less blunt and crooked near the tip, when grown continually in unchanged distilled water. In tap water the roots were long, slender, firm and usually straight. The lateral roots appeared later in tap water than in distilled water.

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